REMARKS

Claims 1-17 are pending, claim 17 having been withdrawn by the Examiner pursuant to Applicant's restriction election.

Applicant herein cancels claim 18 without prejudice.

The Examiner has rejected claims 1-6 and 14-17 under 35 U.S.C. § 103(a), as being allegedly unpatentable over *Elsas* et al. (U.S. Patent No. 6,207,387) in view of either Ehrlich (Bicheimica et Biophysica Acta 395:109-119, 1975) or Hua (Gov. Rep. Announce. Index US, 88, No.18, Abstract No. 847,050, 1988), and in further view of either Tyagi et al (U.S. Patent No. 6,150,097, Nov. 2000) or Coull (U.S. Patent No. 6,355,421, March 2002). Applicant respectfully traverses this rejection as described herein.

The Examiner has rejected claims 7, 10, 12 and 13 under 35 U.S.C. § 103(a), as being allegedly unpatentable over *Elsas* in view of either Ehrlich or Hua, and in further view of either Tyagi et al or Coull, and further in view of Herman et al. (U.S. Patent 6,265,171, July 2001). Applicant respectfully traverses this rejection as described herein.

The Examiner has rejected claims 7 and 8 under 35 U.S.C. § 103(a), as being allegedly unpatentable over *Elsas* in view of either Ehrlich or Hua, and in further view of either Tyagi et al or Coull, and further in view of Kay et al. (Leukemia and Lymphoma, 24:211-220, 1997). Applicant respectfully traverses this rejection as described herein.

Rejections under 35 U.S.C. § 103(a)

Elsas et al, in view of Ehrlich or Hua, and in further view of Tyagi or Coull

The Examiner has rejected claims 1-6 and 14-17 under 35 U.S.C. § 103(a), as being allegedly unpatentable over *Elsas* et al. (U.S. Patent No. 6,207,387) in view of either Ehrlich (Bicheimica et Biophysica Acta 395:109-119, 1975) or Hua (Gov. Rep. Announce. Index US, 88, No.18, Abstract No. 847,050, 1988), and in further view of either Tyagi et al (U.S. Patent No. 6,150,097, Nov. 2000) or Coull (U.S. Patent No. 6,355,421, March 2002).

Applicant respectfully traverses this rejection as described herein, based on the fact that no

prima facie case of obviousness is supportable with these asserted references, alone or in combination. The asserted references actually *teach away* from the presently claimed aspects of the invention.

The present claims:

The presently claimed aspects of the invention are drawn to methods for detecting methylated nucleic acids comprising: contacting (hybridizing) a target nucleic acid sample suspected of containing methylated nucleotides with at least one oligonucleotide probe (molecular beacon-type) comprising first and second stems having fluorophore and a quencher moieties, respectively, and loop sequence having a region of nucleotides complementary to at least a region of the nucleic acid that is susceptible to methylation, wherein the nucleotides forming the first stem are capable of moving into spatial proximity with the nucleotides forming the second stem when the probe is dissociated from the nucleic acid sample; altering the hybridization conditions such that the oligonucleotide probe dissociates from unmethylated nucleic acids but remains hybridized to methylated nucleic acids; and measuring the change in fluorescence, wherein an increase in fluorescence indicates methylated nucleotides in said nucleic acid sample.

Significantly, the claimed aspects encompass the novel conception and appreciation that the oligonucleotide probe melting temperature *differential* between a methylated and unmethylated target sequences), is sufficient for effective detection using a target specific 'loop' sequence in the context of a molecular beacon type probe. That the melting temperature differential would reasonably have such a utility or sufficiency is not suggested by the asserted art and teachings, alone or in combination, and even if there were a suggestion to combine the references and teachings as urged by the Examiner, there could have been no reasonable expectation of success.

The asserted references:

As construed by the Examiner, Elsas teaches detection of mutations by determining the melting temperature between oligonucleotide probes and amplified genomic DNA sequences.

Significantly, however, Elsas do not teach molecular beacon type probes, and do not teach application to methylated DNA nor thermodynamic characteristica of methylated DNA. Moreover, the melting temperature teachings are limited to detecting sequences differing by at least one nucleotide. Therefore, differential melting in Elas is not based on a simple base modification (as in the present case), but is rather based on the presence of at least one base **mismatch** between the probe and target in the detected target sequence. Such Tm differences, as recognized by those in the relevant art, are rather substantial. For example, in Elsas' Example 3, the Tm differential between the wild-type and the galactosemia-positive mutant (Q 188R) allele is 9°C (i.e., the Tm for the hybrid of amplified mutant DNA with the wild-type probe is 56°C, whereas the Tm for the hybrid of amplified mutant DNA hybrid with the mutant allele is 65°C (Elsas at column 11, lines 5-11).

As construed by the Examiner, Erlich teaches that the melting temperature differential between Xanthomonas phage XP-12 DNA containing 5-methylcytosine in **ALL** cytosine positions (i.e., all positions on both strands; in fact, it is the only DNA known to have all cytosines methylated) and normal DNA with the same adenine plus thymine percentage is 6.1°C. Significantly, however, Ehrlich does not compare XP-12 DNA with unmethylated XP-12 DNA, and does not teach the degree/amount/extent that one, or a limited number of methylated cytosines would contribute to such a differential Tm. Ehrlich, therefore, teaches nothing about efficient methods for the detection of methylated genomic sequences, and further teaches nothing about molecular beacon-type probes or the effective use thereof in the presently claimed context.

Similarly, Hua teaches that the melting temperature of completely methylated **Z**-DNA is 7K higher than unmethylated **B**-form DNA. Significantly, however, Hua is not just comparing the effects of methylation versus non-methylation, but rather is comparing the methylated form of one DNA structure with the unmethylated form of another DNA structure under very specific salt conditions. Therefore, it is impossible to distinguish methylation effects on Tm from DNA structure effects on Tm. Additionally, Hua teaches differential Tm in the context of complete methylation, and therefore, aside from the structural differences in the compared DNAs, teaches

nothing about the degree/amount/extent that one, or a limited number of methylated positions would contribute to such a differential Tm. Hua, therefore, teaches nothing about efficient methods for the detection of methylated genomic sequences, and further teaches nothing about molecular beacon-type probes or the effective use thereof in the presently claimed context.

Tyagi et al., consistent with the Examiners interpretation, "discovered that in certain Molecular Beacon probes, a pair of labels "touches" when the probe is not hybridized to a target," so "that the absorption spectrum is significantly altered." (Tygai at column 5 lines 31-34). Therefore, Tyagi teaches quenching in the context of FRET pairs. Tyagi, however, teaches nothing about efficient methods for the detection of methylated genomic sequences, and further teaches nothing about the effective use of molecular beacon-type probes in the presently claimed context of detection of methylated nucleic acid sequences.

Finally, Coull et al., teach peptide nucleic acid (non-naturally occurring polyamide) molecular beacons that have advantageous properties (less susceptible to degradation, etc.). Coull, however, teaches nothing about efficient methods for the detection of methylated genomic sequences, and further teaches nothing about the effective use of molecular beacon-type probes in the presently claimed context of detection of methylated nucleic acid sequences.

Applicable law:

To establish a *prima facie* case of obviousness there must be: (i) a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (ii) a reasonable expectation of success; and (iii) the prior art reference(s) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure (*In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991); and see MPEP §§ 2143-2143.03). Therefore, to support a conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner

must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references. Moreover, there can be no reasonable expectation of success where the art, alone or in combination, *teaches away* from the invention.

As discussed herein above, applicant respectfully contends that no *prima facie* case of obviousness can be made in the present case, because there is no suggestion in Elsas, alone or in combination with any of the other cited references, to provide for the instant novel methods, which provide for effective detection of methylated nucleic acid sequences based on the use of molecular beacon-type probes in the context of Tm measurements. Applicant's invention is therefore novel and non-obvious.

Specifically, according to Applicant's arguments already of record that are reaffirmed and reasserted herein, the asserted art alone or in combination simply does not teach or suggest the claimed combination, and no such teaching or suggestion in these references has in fact been cited by the Examiner. Moreover, *arguendo*, even if there were such suggestions to combine, there would have been no reasonable expectation of success in applying the teachings Ehrlich and Hua (with respect to the differences in Tm between methylated and unmethylated DNA) to the teachings about allele discrimination using molecular beacon-type probes to arrive at the presently claimed invention.

As discussed above in detail with respect to these references, alone or in combination, there is no teaching or embodied expectation as to the degree/extent/amount that one or a limited number of cytosine methylations would make to a differential Tm in the presently claimed context. The cited references teach completely methylated sequences **on both strands**, and comparisons between DNAs having **different structures**. Moreover, as discussed above, Elsas teaches at least one base mismatch, associated with a significant (9°C) effect on Tm. Based on the cited references, it would certainly not have been obvious that the Tm differentials associated with cytosine methyation would have been sufficient to enable effective detection of methylated nucleic acids in the context of Tm measurement using molecular beacon-type probes. This is

particularly true when one considers that the presently claimed molecular beacon 'loop' sequences are not themselves methylated, so that at best only one strand of the resulting hybrid DNA (i.e., the detected genomic strand) is methylated. Therefore, if anything, Elsas, Ehrlich and Hua collectively *teach away* from the present invention by teaching that the differential effects of methylation on Tm are likely too small to provide effective detection assays. Therefore, not only would there be no reasonable expectation of success with respect to the sufficiency of the presently claimed methods with respect to double-stranded target DNA where both DNA strands would be methylated, there certainly would have been no reasonable expectation of success where only a single target strand of the hybid (and not the 'loop' sequence) would be methylated.

Applicant, therefore, respectfully requests withdrawal of the Examiner's 35 U.S.C. § 103(a) rejections of claims 1-6 and 14-17.

Elsas et al, in view of Ehrlich or Hua, in further view of Tyagi or Coull, and in further view of Herman et al.

The Examiner has rejected claims 7, 10, 12 and 13 under 35 U.S.C. § 103(a), as being allegedly unpatentable over *Elsas* in view of either Ehrlich or Hua, and in further view of either Tyagi et al or Coull, and further in view of Herman et al. (U.S. Patent 6,265,171, July 2001).

The Examiner states that while "Elsas, Ehrlich, Hua, Tyagi or Coull do not teach detecting methylation in GST pi or calcitonin which is differentially methylated in cancer versus normal state," Herman nonetheless "teaches numerous genes which are differentaiilly methylated at CpG islands in neoplastic versus normal tissue," and that "CpG island differential methylation can be detected in prostate cancer."

Applicant respectfully traverses this rejection based on the arguments discussed in detail above with respect to the Examiner's rejection of claims 1-6 and 14-17. The combination of cited references does not anticipate independent claim 1 or render it obvious.

Applicant, therefore, respectfully requests withdrawal of the Examiner's 35 U.S.C. § 103(a) rejections of claims 7, 10, 12 and 13.

Applicant notes that the Examiner, despite the reference to differentially methylated CpG island sequences and prostate cancer, has made no specific allegation with respect to the teachings, if any, of Herman with respect to differential GSTpi and calcitonin methylation and prostate cancer.

Elsas et al, in view of Ehrlich or Hua, in further view of Tyagi or Coull, and in further view of Kay et al.

The Examiner has rejected claims 7 and 8 under 35 U.S.C. § 103(a), as being allegedly unpatentable over *Elsas* in view of either Ehrlich or Hua, and in further view of either Tyagi et al or Coull, and further in view of Kay et al. (Leukemia and Lymphoma, 24:211-220, 1997). Applicant respectfully traverses this rejection as described herein.

The Examiner states that while "Elsas, Ehrlich, Hua, Tyagi or Coull do not teach differential methylation in Myf-3 which is differentially expressed in cancer versus normal state," Kay nonetheless teaches "detecting methylation in Myf-3, which is differentially expressed in cancer versus a normal state" ("hypermethylated in non-Hodgkins lymphoma").

Applicant respectfully traverses this rejection based on the arguments discussed in detail above with respect to the Examiner's rejection of claims 1-6 and 14-17. The combination of cited references does not anticipate independent claim 1 or render it obvious.

Applicant, therefore, respectfully requests withdrawal of the Examiner's 35 U.S.C. § 103(a) rejections of claims 7 and 8.

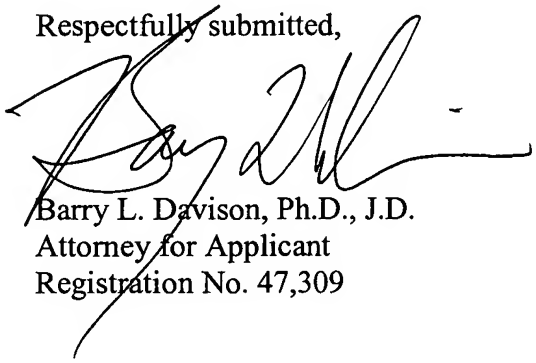
CONCLUSION

The asserted art, alone or in combination, neither anticipates nor renders obvious applicant's presently claimed inventive subject matter.

In view of the foregoing amendments and remarks, applicant respectfully requests entry of the present Response and Amendment, and allowance of all claims as presented and amended herein.

The Examiner is encouraged to phone applicant's attorney, Barry L. Davison, to resolve any outstanding issues and expedite allowance of this application.

Respectfully submitted,



Barry L. Davison, Ph.D., J.D.
Attorney for Applicant
Registration No. 47,309

Davis Wright Tremaine LLP
2600 Century Square
1501 Fourth Avenue
Seattle, Washington 98101-1688
Telephone (206) 628-7621
Facsimile (206) 628-7699